Microbial Reduction of Cr^{6+} on Mineral Surfaces in Columbia Basalt Rocks: A Synchrotron FTIR Study

Hoi-Ying N. Holman, Dale L. Perry, Michael C. Martin, Wayne R. McKinney, and Jennie C. Hunter-Cevera
Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720

Industrially produced and discharged hexavalent chromium, Cr^{6+}, occurs in the environment in the highly water soluble forms of the divalent oxyanions chromate $(CrO_4)^{2-}$ and dichromate $(Cr_2O_7)^{2-}$ *(1)*. These compounds readily cross cell membranes mainly *via* the sulfate $(SO_4)^{2-}$ active transport system (*2*) and are reduced intracellularly to Cr^{5+} and Cr^{3+}, which then mutagenize DNA by disrupting DNA replication *(3)*. When in the environment in the reduced trivalent form (Cr^{3+}), they tend to form relatively insoluble compounds that often cannot cross cell membranes and thus become significantly less harmful to the same biological systems (*3*). These profound changes in physical, chemical, and toxicological properties following reduction have created significant opportunities to detoxify Cr^{6+} in geological materials (*4*).

There are two contradicting views about hexavalent chromium Cr^{6+} reduction mechanisms (and polyvalent metal ions as a whole) on mineral surfaces. The "biological mechanism" is based on the presence of Cr^{6+}-tolerant and Cr^{6+}-reducing microorganisms detected by conventional microbial techniques in well-sorted and well-mixed geological materials (*5*). The evidence is corroborated with Cr^{6+} reduction to Cr^{3+} measured in batch and flow-through column experiments using similar geologic materials (*6-8*). Recent research indicates that the role of the microbial mechanism is further enhanced by microbially produced macromolecules *(9)*. Through combinations of carboxyl, phosphoryl, and hydroxyl groups, these macromolecules complex polyvalent metal ions and increase the chromium solubility and thus availability to Cr^{6+}-tolerant and Cr^{6+}-reducing microorganisms (*9)*. The alternative "chemical mechanism" suggests that the presence of geological minerals with mixed metal oxides and/or the presence of natural organic molecules can significantly catalyze the redox chemistry of Cr^{6+} and other polyvalent metal ions in geological materials *(10-12).*

In this study, we report systematically observed evidence that provides insight into dominant reduction mechanisms and reduction pathways on mineral surfaces. The microorganisms under study are rock-inhabiting microorganisms in fractured basalt rock systems. They are found in the vadose zone above the rock aquifers within the extensive Columbia basalt flow in the southwestern part of Idaho in the United States. The basalt samples are from the Radioactive Waste Management Complex (RWMC) within the Idaho National Engineering and Environmental Laboratory (INEEL) of the U.S. Department of Energy. This site has been polluted with mixtures of hexavalent chromium, Cr^{6+}**,** together with other inorganic ions, radionuclides, petroleum hydrocarbons, and volatile organic compounds (VOCs) from more than 40 years of US nuclear production activity. The samples were collected and sectioned at the site by members of INEEL.

All samples were fine-grained silicate-containing vesicular basalt. Earlier work indicates that although Columbia basalt rocks are generally quite limited in nutrients and organic carbon,

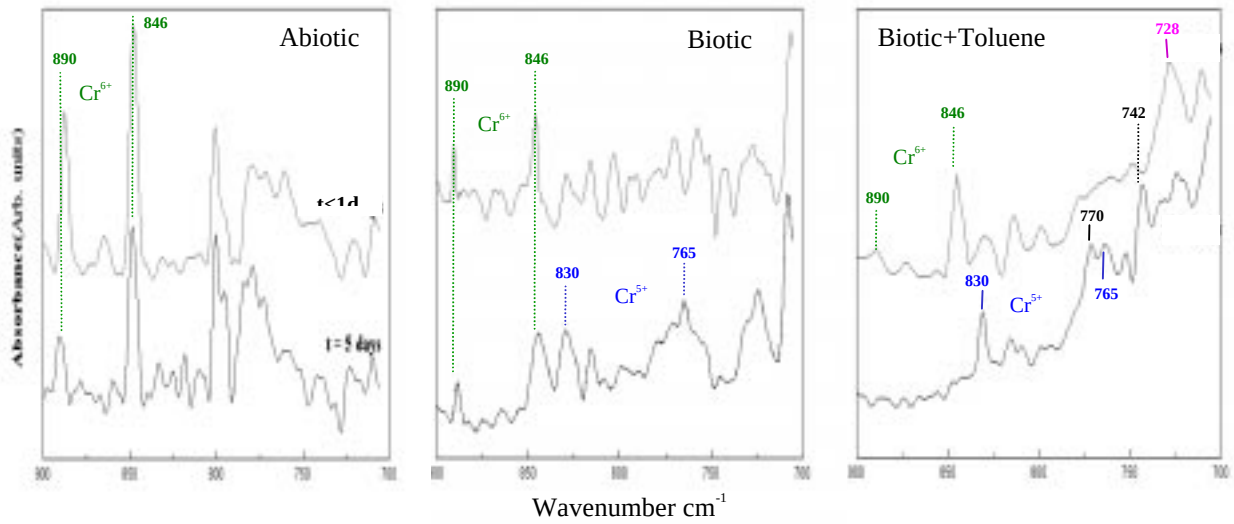

Figure 1: SR FTIR spectra of chromate on magnetite surfaces during the 5-day experiment of (left) abiotic reduction, (middle) biotic reduction in the absence of other organic compounds, and (right) biotic reduction in the presence of toluene vapor (as a model volatile organic compound).

there are dense clusters of microorganisms on the vesicles or on the fractured surfaces (*14*), especially in areas where magnetite is a dominant constituent. Batch studies demonstrated that some of these microorganisms are Cr^{6+}-tolerent and Cr^{6+}-reducing microorganisms (*15,16*). The ability of these rock-inhabiting microorganisms to aerobically reduce Cr^{6+} increased significantly during concomitant biodegradation of a volatile organic compound (VOC) (*15*). Other bacteria with similar abilities to detoxify mixtures of Cr^{6+} and VOCs have been reported elsewhere (*7*).

We used a synchrotron radiation-based (SR) Fourier transform infrared (FTIR) spectromicroscopy beamline (Beamline 1.4.3 at the Advanced Light Source, Lawrence Berkeley National Laboratory) previously described (*17*) to spectroscopically and spatially document the reduction process as it occurred on the basalt surfaces. All FTIR microspectra were recorded in reflectance mode over the 4000-650 cm^{-1} infrared region at 4 cm^{-1} resolution on a Nicolet Magna 760 FTIR-spectrometer coupled to a Nic-Plan IR microscope (*18*). The SR source is at least 200 times brighter at a 10-μm spatial resolution than a conventional black-body IR source. This makes the technique most useful for examining in real time and at high spatial resolution many compounds and organisms at (dilute) environmental concentrations. The distinct IR absorption bands we used as chemical markers are well known (*14, 17, 19-20*).

The experiment began with an introduction of 10-ppm (as chromium) chromate solution onto different magnetite surfaces. Fig. 1 shows recorded SR FTIR spectra for chromium on magnetite surfaces shortly after the experiment's beginning and 5 days after exposure. For magnetite surfaces without living *A. oxydans* cells (abiotic), we observed no statistically significant changes in either the IR absorption intensity or characteristic band shapes during the 5-day period. For magnetite surfaces with living *A. oxydans* cells (biotic) but without the benefit of biodegradation of toluene, the only large change in the spectral region shown was the moderate decrease in the absorption intensity of the Cr^{6+} compound $(CrO_4)^{2-}$ vibrational mode at 846 cm^{-1} (*19*). A weaker band at 830 cm^{-1} appeared and was accompanied by an even weaker

one at 765 cm^{-1}. These bands are reminiscent of either the IR-active stretching frequencies of chromium-oxygen in chromium-carboxylate and chromium-amino acid complexes (*21*), or the vibrations of oxalate (*22*) and catecholate (*23*) anions that both can complex with and/or reduce transition metal ions. All of these organic classes of compounds have been documented (*24-25*) as being natural products of microbial activities, although it is difficult to establish precisely which complexes are being observed (*27*).

Magnetite surfaces with living *A. oxydans* cells and the added toluene vapor (biotic + toluene), had all infrared absorption bands in the spectral region displayed significant changes in both intensity and characteristic band shape during the 5-day period. In particular, the absorption intensities of the 846 cm^{-1} and 728 cm^{-1} modes, which are assigned to $(CrO_4)^{2-}$ and toluene (*19*), disappeared. At the same time, a new, strong band at 830 cm^{-1} appeared and was accompanied by a sharp but weaker satellite at 765 cm^{-1}. They are again associated with possible intermediate Cr^{5+}. The additional new band at 742 cm^{-1}, accompanied by a sharp and slightly weaker satellite at 770 cm^{-1}, is possibly associated with catechol (*28*), one of toluene's many biodegradation products (29).

The spatial distributions of IR absorption associated with protein Amide II of *A. oxydans* (1550 cm^{-1}), chromate (846 cm^{-1}), and toluene (728 cm^{-1}) were mapped and are presented in Fig. 2. The close link between the microbial reduction of Cr^{6+} and the biodegradation of toluene is evident. It also reveals that the transformation of chromate and toluene during the 5-day experimental period is controlled spatially by the microorganisms. The lack of significant chromate reduction on surfaces that are free of, or low in, bacterial density supports our thesis that the microbial reduction mechanism is significant even on surfaces of mixed iron oxides.

Similar SR FTIR mapping experiments were conducted on composite mineral surfaces in basalt rocks during four months under conditions typical of the vadose environment at the INEEL site. Spatial distributions of vibrational frequencies associated with intrinsic microorganisms (1550 cm^{-1}), chromate $(CrO_4)^{2-}$ (846 cm^{-1}), reduced Cr^{5+} compounds (830 cm^{-1}), and Cr^{3+} compounds (810 cm^{-1}) were mapped at the end of the second week and the end of the fourth month. At the end of the second week no Cr^{3+} compound was detected. At the end of the fourth

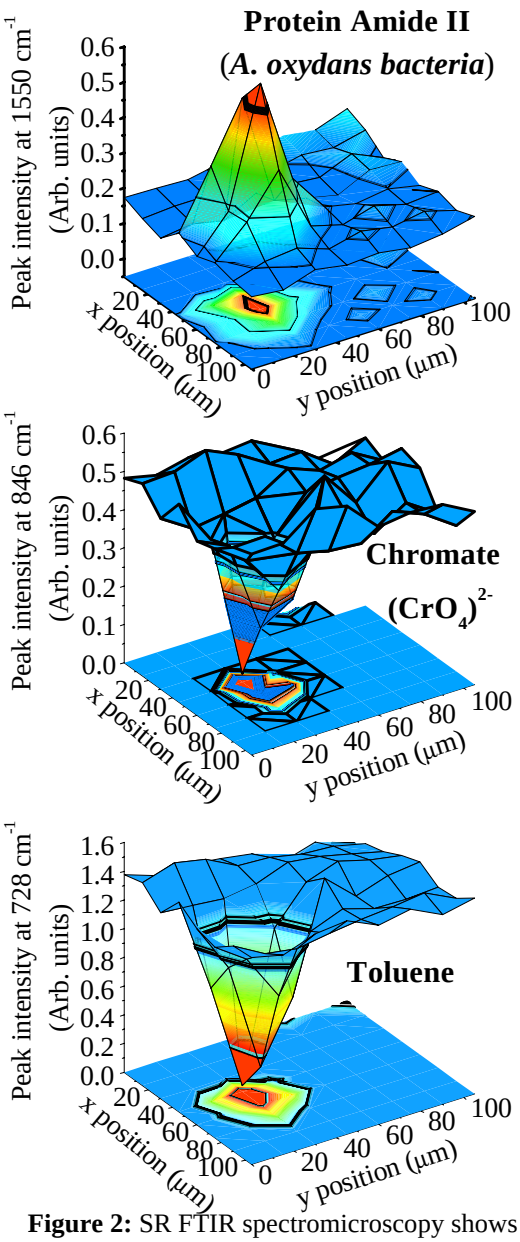

Figure 2: SR FTIR spectromicroscopy shows the spatial distribution of (top) *A. oxydans*, (middle) chromate, and (bottom) toluene as measured by their spectral signatures.

month, spatial distributions of the same vibrational frequencies (Fig. 3) demonstrate a correlation between the Cr^{6+} reduction to Cr^{3+} and the distribution of microorganisms. This implies that a four-month incubation time had selectively enriched for the successful growth of Cr^{6+}-tolerant and Cr^{6+}-reducing native microorganisms.

The reduced Cr^{3+} compounds in the present experiments have been confirmed by x-ray absorption fine structure (XAFS) spectroscopy obtained on Beamline 10.3.2 at the Advanced Light Source (*30*). Measurements done at the same location as the peak in Fig. 3 shows a Cr x-ray absorption edge structure and lack of pre-edge peak that is consistent with Cr^{3+} compounds. All microorganisms on the sample surfaces were killed by the x-ray irradiation during the XAFS experiment. The SR FTIR and the XAFS experiments were repeated after three months and indicated no Cr^{3+}-reoxidation. This implies that the reduced Cr^{3+} is relatively stable under normal atmospheric conditions, which is consistent with results previously reported (*1*).

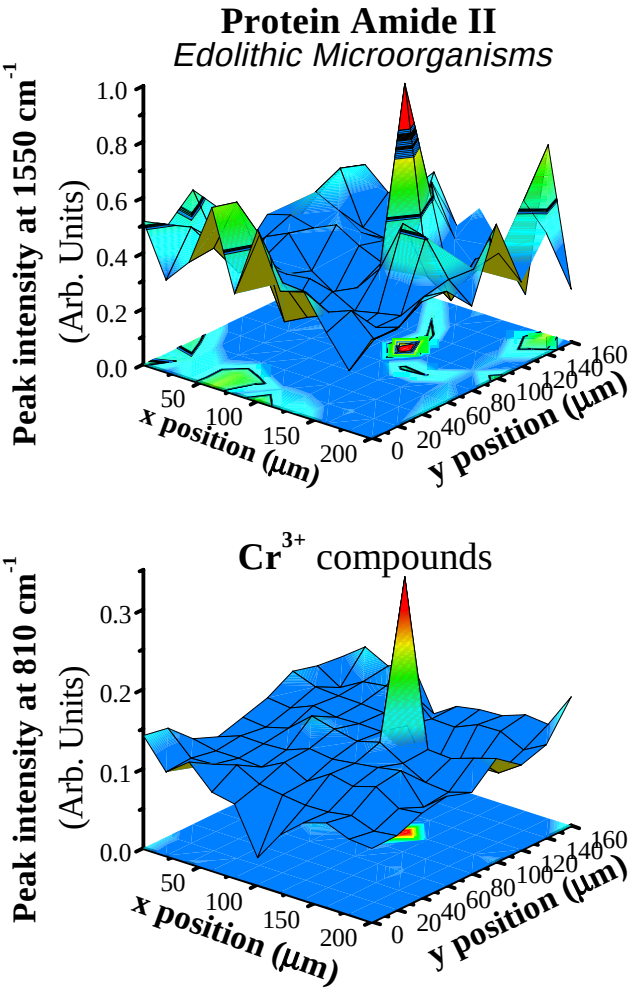

Figure 3: Distribution of indigenous microorganisms (top) and the Cr^{3+} compounds (bottom) after a 4-months Cr^{6+}-microbe-basalt experiment.

Summary

These SR FTIR spectromicroscopic observations have nondestructively monitored and elucidated, for the first time to our knowledge, the transformation and mechanisms of toxic Cr^{6+} on mineral surfaces. It has demonstrated that the microbial reduction of Cr^{6+} is important on magnetite surfaces and on surfaces of Columbia basalt rocks that are rich in magnetite. The Cr^{6+} reduction reaction accelerates during concomitant biodegradation of toluene (a common co-contaminant), which further confirms the significance of biological activities. Our spatially resolved SR FTIR spectromicroscopy further shows that on magnetite, Cr^{6+} is reduced only in the presence of the isolated microorganisms. Time resolved studies indicate that the reduction of Cr^{6+} to Cr^{3+} proceeds at least as a two-step reaction with Cr^{5+} compounds as probable intermediate products. The reduced Cr^{3+} compounds were observed to be stable for many months even after the microorganisms were killed. Thus mutagenic Cr^{6+} pollutants in the environment can be biotransformed into less mobile, less toxic, and stable compounds. One must consider the significant role of microorganisms when designing and implementing new and environmentally benign remediation technologies for the cleanup of mixed waste sites.

References and notes:

1. H. Ohtake and S. Silver, in *Biologic Degradation and Bioremediation of Toxic Chemicals*, G.R. Chaudhry, Ed. (Chapman & Hal, New York, 1994), pp.403-415.
2. E.T. Snow, *Health Perspect*., **92**, 75 (1991).
3. E. Nieboer and A.A. Jusys, in *Chromium in the Natural and Human Environments*, J.O. Nriagu and E. Nieboer, Eds. (John Wiley & Sons, New York, 1988), pp.21-80.
4. D.R. Lovely and J.D. Coates, *Current Opinion in Biotechn*., **8**, 285 (1997).
5. C.E. Turick *et al*., *Applied Microbiol. Biotechnol*., **44**, 683 (1996)
6. M.E. Losi *et al*., *Environ. Toxicol. And Chem*., **13**, 11, 1727 (1994).
7. H. Shen *et al*., *Biotechnol. And Bioeng.* **52**, 357 (1996).
8. E.M.N. Chirwa and T.-T. Wang, *Enivron. Sci. Technol*., **31**, 1446 (1997).
9. D. Ahmann, *Society of Ind. Microbiol.*, **47**, 5, 218 (1997).
10. G. Bidoglio *et al*., *Geochimica et Coschimica Acta*, **57**, 10, 2389 (1993).
11. T. Guo *et al*., *Environ. International*, **23**, 3, 305 (1997).
12. B. Deng and A.T. Stone, *Environ. Sci. Technol*., **30**, 2484 (1996).
13. W.T. Griffin et al., in *The Microbiology of the Terrestrial Deep Subsurface*, P.S. Amy and E.L. Haldeman, Eds. (CRC Lewis Publishers, New York, 1997), pp.23-44.
14. H.-Y. N. Holman *et al*., *J. of Microbiol. Methods*, in press, 1999.
15. Cr^{6+}-tolerant microorganisms were isolated from rock samples using basalt extract-yeast extract agar enriched with 10 mg/L^{-1} chromate (as chromium). The identity of the microorganisms is based on colony and cellular morphology and fatty acid methyl ester (FAME) analysis.
16. American Public Health Association, in *Standard Methods for the Examination of Water and Wastewater, 18th Ed.*, (Victor Graphic, Baltimore, 1992), pp.3.59-3.60.
17. H.-Y. N. Holman *et al*., *in Applications of Synchrotron Radiation Techniques to Materials Science IV, MRS Proceedings*, **524**, 17 (1998).
18. Absorption bands from water vapor on the specimens interfere with the quality of recorded SR FTIR spectra and especially complicate the identification of the biomolecule markers in the 1800 – 1500 cm^{-1} region. To minimize the water vapor effect, all specimens were kept overnight in sterile dry air before the FTIR measurement began. That this moderate drying was sufficient to minimize interference from the residual water in the sample was confirmed when water vapor absorption bands and their second derivative bands in the 1800 – 1500 cm^{-1} region did not hinder the identification of the biomolecule markers.
19. H.H. Schmidtke and D. Garthoff, *Helv. Chim. Acta*, **50**, 1631 (1967).
20. C. Engelter and D.A. Thornton, *J. Mol. Struct*., **33**, 119 (1976).
21. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds, 4th Ed*, (John Wiley & Sons, New York, 1986).
22. J. Fujita et al., *J. Chem. Phys*., **36**, 2, 324 (1962).
23. W.P.Griffith *et al*., *J. Chem. Soc. Dalton Trans*., 1125 (1986).
24. J. Szarapinska-Kwaszewska *et al*., *Med. Doswi. I Mik*., **50**, 1-2, 9 (1998).
25. N.C. Russell *et al*., *Antarctic Sci*., **10**, 1, 63 (1998).
26. It should be remembered that the infrared spectra of metal-organic compounds can vary even within the same metal-organic pair in their different complexes (*27*), thus making it very difficult to establish exactly which complex one is observing. This is in addition to the shift of some vibrational lines due to hydrogen bonding, molecular hydration, and other effects.
27. E. Faulques *et al*., *Spectrochim. Acta*, **54**, 869 (1998).
28. C.J. Pouchert, *The Aldrich Library of FT-IR Spectra*, (Aldrich Chemical Company, 1985), Vol.1, pp.931-970.
29. C.E. Cerniglia, in *Petroleum Microbiology*, R.M. Atlas Ed., (Macmillan, New York, 1984), pp.99-128.
30. The micro-x-ray analysis was conducted by Dr. Geraldine Lamble at ALS Beamline 10.3.2.

We thank Dr. Geraldine Lamble for performing the micro-x-ray analysis; Drs. Terry Hazen, Tamas Torok, and Arthur Robinson for reviewing the manuscript. This work was performed with support by the Directors, Office of Energy Research, Offices of Health and Environmental Sciences, Biological and Environmental Research Program and work at the Advance Light Source is supported by Basic Energy Sciences, Materials Science Division, United States Department of Energy under Contract No. DE-AC03-76SF00098.